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Predicted co-receptor tropism and sequence characteristics of China HIV-1 V3 loops: implications for the future usage of CCR5 antagonists and AIDS vaccine development

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Summary
Background: The co-receptor tropism of any given HIV-1 isolate is closely associated with the progression of AIDS. Understanding the co-receptor tropism and genetic diversity of circulating HIV-1 strains is critical for AIDS treatment and vaccine development.
Methods: All available China HIV-1 V3 sequences with known subtypes/circulating recombinant forms (CRFs) and transmission routes were retrieved from the Los Alamos HIV Sequence Database. HIV-1 co-receptor tropism was predicted using online tool HIV-1 PhenoPred.
Results: All C/CRF07_BC/CRF08_BC strains appeared to use CCR5 for cell entry (R5 strains), while 61.1% of subtype B and 38.7% of CRF01_AE were also R5, indicating a higher prevalence of R5 (76.9%) than X4. The prevalence of R5 remained relatively stable over the different sample years regardless of C/CRF07_BC/CRF08_BC, B, or CRF01_AE subtypes. The co-receptor usage of HIV-1 appeared to be associated with the different subtypes, rather than transmission route. Furthermore, the V3 sequences of C/CRF07_BC/CRF08_BC were more genetically homogeneous relative to both subtypes B and CRF01_AE.
Conclusions: The higher prevalence of R5 and higher level of homogeneity of V3 sequences in C/CRF07_BC/CRF08_BC suggest that CCR5 antagonists will be promising drugs for future AIDS treatment in China, and that circulating R5 strains are valuable candidates for AIDS vaccine development.

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Introduction

The entry of HIV-1 into human target cells requires a first receptor and a second (co-) receptor. Two human chemokine receptors, CCR5 and CXCR4, are usually used as co-receptors for HIV-1 entry,[1] and different HIV-1 isolates use different co-receptors. HIV-1 R5 and X4 strains use CCR5 and CXCR4 as co-receptors, respectively, and R5X4 strains use both of them.[1] R5 strains often have a slow replication rate and low virulence in peripheral blood mononuclear cells (PBMC), whereas X4 variants appear to replicate rapidly and have high virulence.[2,3]

R5 strains are the dominant viral phenotype for HIV-1 transmission,[4,5] and are often detected during the early stages of HIV-1 infection, even throughout infection.[6,7] X4 strains evolve from R5 variants, possibly via the R5X4 intermediates, and typically emerge during the later stages of infection. The emergence of X4 strains is usually accompanied by an accelerated decrease in CD4+ T cell counts, implying an association between AIDS progression and the emergence of CXCR4-using strains.[6,8–10] HIV-1 co-receptor tropisms in patients determine the optimal time of antiretroviral therapy initiation and optimal treatment strategies.

A large-scale survey on the molecular epidemiology of HIV showed that there are eight subtypes or circulating recombinant forms (CRFs) circulating in China, namely A, B′, B, C, CRF07_BC, CRF08_BC, CRF01_AE, and CRF02_AG.[11] However, co-receptor tropisms of these HIV-1 strains have not been fully characterized. Understanding the co-receptor tropism and genetic diversity of circulating HIV-1 strains is critical for AIDS treatment and vaccine development in China.

Materials and methods

China V3 sequence and co-receptor prediction

The V3 loop of HIV-1 gp120, a disulfide-linked loop of approximately 35 amino acids, plays a dominant role in the determination of viral co-receptor usage and phenotype.[12,13] In order to investigate HIV-1 co-receptor tropism, all available HIV-1 V3 nucleic acid sequences of the five major subtypes/CRFs (including 411 B, 28 C, 106 CRF01, 325 CRF07, and 103 CRF08) circulating in China were retrieved from the Los Alamos HIV Sequence Database (http://www.hiv.lanl.gov/content/hiv-db/mainpage.html) in September 2008. The co-receptor usages were predicted using our online prediction tool (http://bioinfo.gnway.net/HIV-1/PhenoPred.php),[14] which performs better for all HIV-1 subtypes than previous methods PSSM and Geno2pheno, and can predict the R5X4 intermediates (unpublished data). For cloned V3 sequences, the prediction accuracies of our tool are 99.2% for R5 and 88.3% for X4 (including R5X4). For clinical isolates, it has the same prediction accuracy (90%) for X4 strains at the same specificity (90%) level for X4 as Geno2pheno, which is the most robust tool for CXCR4 usage prediction. The determination of the emergence of CXCR4-using strains plays a key role in the initiation of clinical highly active antiretroviral therapy (HAART). To improve the prediction of R5X4 and X4 strains, all predictions were performed at the 90% specificity level for X4 strains. Furthermore, for V3 sequences containing ambiguous bases, all possible translations were respectively predicted. If one or more of these translations were predicted as R5X4 or X4, this sample would be classified as R5X4 or X4.

V3 sequence characteristic analyses

To show the V3 loop characteristic of three major subtypes/CRFs, the consensus sequence of each subtype or CRF was obtained using WebLogo (http://weblogo.berkeley.edu/logo.cgi).[15] Because the V3 regions of CRF07 and CRF08 originate from subtype C, both CRFs were referred to as subtype C in the sequence characteristic analysis.

Statistical analysis

All statistical comparisons between any two different groups in this study were conducted using a two-sided Fisher's exact test (GraphPad Prism version 4.03 (Windows demo)).

Results

HIV-1 co-receptor tropism is associated with different subtypes/CRFs

The predicted co-receptor tropisms of five HIV-1 subtypes/CRFs are shown in Table 1. For subtype B, the proportions of R5, R5X4, and X4 were 61.1%, 26.5%, and 12.4%, respectively. A similar distribution of co-receptor tropism was found for CRF01_AE, with 38.7% R5, 39.6% R5X4, and 21.7% X4. Both B and CRF01_AE strains analyzed in this study were isolated during 1992–2005. Because of the association of R5X4/X4 strains with AIDS progression,[6,9,10] HIV-1 co-receptor tropism was compared between the different sample years. Results

Table 1 Co-receptor tropisms of China HIV-1 strains from various subtypes/CRFs and different transmission routes

Co-receptor tropism	HIV-1 subtypes/CRFs					Transmission route		
	B	C	CRF01_AE	CRF07_BC	CRF08_BC	IDU	UBPC	Heterosexual
R5	251	28	41	325	103	378	109	4
R5X4	109	0	42	0	0	23	51	4
X4	51	0	23	0	0	2	29	7
Total	411	28	106	325	103	403	189	15

IDU, injecting drug use; UBPC, unsafe blood- and plasma-collection.
Note: The IDU group includes two subtype B sequences, 34 CRF01_AE sequences, seven CRF15_01B sequences, and 360 CRF07/CRF08/C sequences; the UBPC group includes 188 subtype B sequences and one subtype D sequence; the heterosexual transmission group includes 14 CRF01_AE sequences and one CRF07_BC sequence.

Table 2 Co-receptor tropisms of HIV-1 B and CRF01_AE strains isolated during different sample years

Co-receptor tropism	Subtype B					CRF01_AE			
	1992—1998	2000—2002	2003	2004	2005—2006	1992—1996	1997—1998	2000	2005—2006
R5	15 (71.4)	6 (75)	98 (55.4)	14 (73.7)	10 (76.9)	5 (26.3)	6 (54.5)	9 (40.9)	3 (23.1)
R5X4	6 (28.6)	2 (25)	51 (28.8)	3 (15.8)	2 (15.4)	14 (73.7)	5 (45.5)	9 (40.9)	4 (30.8)
X4	0	0	28 (15.8)	2 (10.5)	1 (7.7)	0	0	4 (18.2)	6 (46.1)
Total	21	8	177	19	13	19	11	22	13

Note: Percentages are shown in parentheses.

showed that no significant difference in co-receptor usages was observed between the different sample years with regard to B ($p \geq 0.1484$) or CRF01_AE isolates ($p \geq 0.2060$) (Table 2). In particular, high proportions of R5X4/X4 were found to have occurred in the earlier sample years (28.6% for subtype B during 1992—1998 and 73.7% for CRF01_AE during 1992—1996), with continuing prevalence throughout the whole epidemic (Table 2), indicating that the high prevalence of R5X4/X4 in subtypes B and CRF01_AE was not ascribed to longer established infection. These observations are consistent with the fact that in HIV-1 subtype B, and even in CRF01_AE, CXCR4 usage ranges from about 20% in early infection to about 50% in advanced disease.[16—18]

Like subtype B and CRF01_AE, the isolates from subtype C, CRF07_BC, and CRF08_BC also had a long sample period from 1996 to 2005. However, all C/CRF07_BC/CRF08_BC strains appeared to be CCR5-using (R5) and had non-syncytium-inducing (NSI) phenotype. Because CRFs 07 and 08 are recombination viruses that have mosaic genomes originated from the HIV-1 subtype C genomic backbone by inserting several subtype B segments,[19,20] the V3 sequences of CRFs _BC are identical to subtype C, and might be regarded as subtype C. The finding of all C/CRF07/CRF08 strains being R5 is consistent with previous observations of an overwhelming predominance of R5 viruses in the whole HIV-1 subtype C infection, even in progression to AIDS,[21—23] and a lack of the co-receptor switch in subtype C-related strain infection.[22,24,25] Furthermore, a significantly higher proportion of R5 strains in C/CRF07_BC/CRF08_BC (100%) than in subtype B (61.1%) and CRF01_AE (38.7%) ($p < 0.0001$) indicates that there are

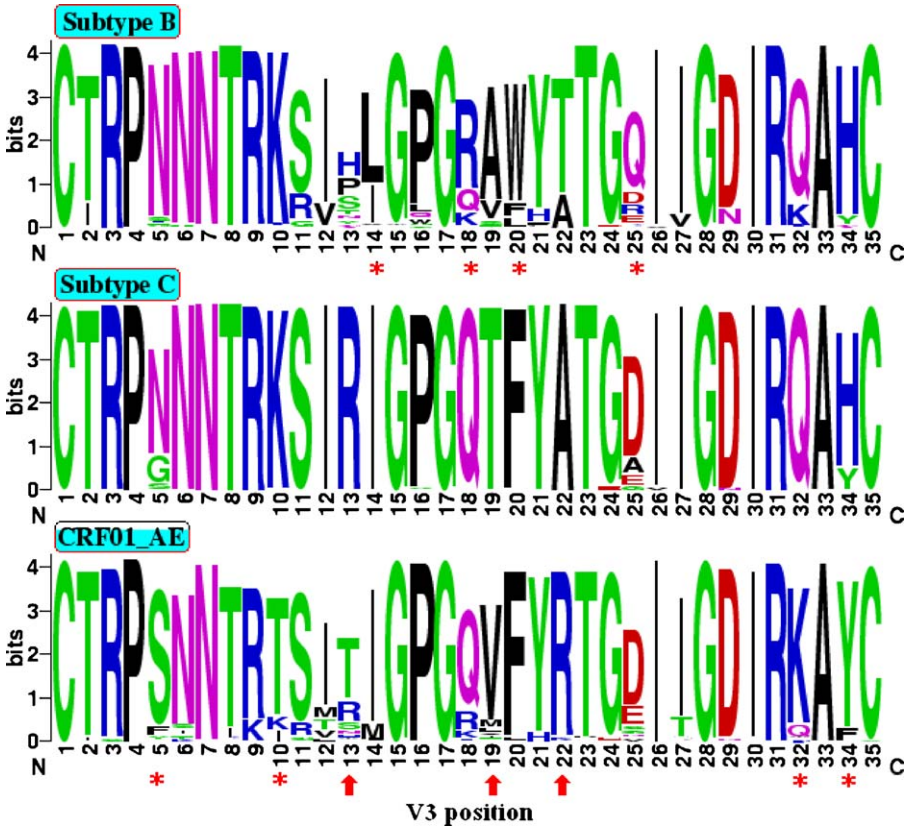

Figure 1 Sequence logos of China V3 residue sequences used in this study. The character and size of each logo represent the proportion of an amino acid at the specific site. Subtype C includes CRF07_BC, CRF08_BC, and other B′C inter-subtypes recombinants. The sequence logos of subtypes B, C, and CRF01_AE were based on 413, 460, and 126 V3 amino acid sequences, respectively. The red arrows highlight the sites of V3 loop that are obviously different between the three major subtypes/CRFs, and the red stars indicate that the site occurring in one subtype is obviously different from that of the other two subtypes/CRFs.

subtype-specific differences in HIV-1 co-receptor usage (Table 1).[23]

No association between HIV-1 co-receptor tropism and transmission route

When taking transmission route into account, the majority of injecting drug use (IDU)-associated viruses appeared to be CCR5-using (R5) (93.8%), whereas the majority of unsafe blood- and plasma-collection (UBPC)-associated (42.3%) and heterosexual transmission-associated (73.3%) viruses were R5X4/X4 strains (Table 1). As the majority (89.3%) of IDU-associated viruses belonged to C/CRF07_BC/CRF08_BC and almost all UBPC-associated viruses (99.5%) were B subtype (Table 1), transmission route appears not to be associated with HIV-1 co-receptor tropism.

Sequence characteristics of China HIV-1 V3 loops

Sequence characteristics of HIV-1 V3 loops from three major subtypes/CRFs circulating in China are shown in Figure 1. The subtype C sequence includes CRF07_BC and CRF08_BC due to their identical V3 sequences. Three V3 sites — 13, 19, and 22 — are obviously different between the three major subtypes/CRFs (Figure 1). Arg, Thr, and Ala, respectively, emerged at the three sites of subtype C V3 loop with a much higher frequency as compared to His/Pro/Ser, Ala, and Thr in subtype B sequences and Thr, Val, and Arg in CRF01_AE sequences. Furthermore, higher frequencies of Leu, Arg, Trp, and Gln were observed respectively occurring at sites 14, 18, 20, and 25 of subtype B, obviously different from the corresponding sites of both subtypes C and CRF01_AE. Another four residues at sites 5, 10, 32, and 34 of CRF01_AE were also obviously different from the other subtypes. It is worth noting that there was no site occurring in subtype C that was obviously different from the other two subtypes/CRFs (Figure 1). In particular, apart from sites 5, 25, and 34, all other sites of subtype C V3 sequences were highly conserved. This suggests that subtype C (including CRF07_BC and CRF08_BC) V3 sequences are more genetically homogeneous relative to both subtype B and CRF01_AE.

Discussion

Current bioinformatics tools have been demonstrated to perform very well in R5 prediction, but relatively weakly in X4 prediction, especially for clinical isolates.[26] In this study, 76.9% of all China isolates were predicted to be R5 using our HIV-1 PhenoPred tool at the 90% specificity level of X4. Prediction at the 90% specificity level of X4 will lead to an underestimate of R5 proportion due to some R5 strains falsely being predicted as R5X4 or X4.[26] When the 99% specificity level of X4 was used, the R5 proportion increased to 86.4%. Therefore, the actual R5 prevalence in China should be higher than 76.9%. Furthermore, a pivotal change in the HIV-1 epidemic in China was found to be associated with a rapid increase in the prevalence of B′C recombination forms (from 30.4% in 1998 to 50.2% in 2003) and a decreasing prevalence of subtype B (from 47.5% in 1998 to 29.1% in 2003).[11] This change further indicates that R5 strains will become overwhelming preponderant in the future. Therefore, instead of traditional HAART, CCR5 antagonists that can block the entry of HIV-1 will be better candidate drugs for present and future AIDS treatment in China.[27,28]

HIV-1 strains isolated during the earliest stages of infection are relatively more homogeneous than those during later stages and exhibit the R5 characteristic.[29] A high degree of homogeneity of R5 viruses appears to have large selective advantages during transmission, implying that HIV-1 infection often begins with a relatively homogeneous R5 population.[29] The finding that all C/CRF07_BC/CRF08_BC strains were R5 viruses and had a higher level of V3 homogeneity strongly suggests that circulating R5 strains with C subtype V3 sequences might be the best vaccine candidates in China and should be taken into consideration in future AIDS vaccine design.

Conclusions

We found that China HIV-1 strains had subtype-specific differences in co-receptor tropism and that the differences were not associated with transmission route. The findings of higher proportions of R5 and higher levels of V3 sequence homogeneity occurring in subtype C and BC inter-subtype recombinants than in subtype B and CRF01_AE, suggest that CCR5 antagonists will be promising drugs for use in place of the traditional HAART in future AIDS treatment in China. Furthermore, it also suggests that circulating China R5 strains with C subtype V3 sequences may be valuable candidates for AIDS vaccine development in China.

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Conflict of interest: No conflict of interest to declare.

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